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6. AUTHOR(S)
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13. ABSTRACT *(Maximum 200 words)*

The encapsulation membranes fabricated from solutions containing Poly(acrylonitrilevinylchloride) (PAN-PVC) (85%) and polyethylene oxide (PEO) (15%) were tested for restoration of circadian activity rhythms to animals whose own SCN has been ablated. This work shows that the transplanted SCN, like neural pacemakers of Drosophilia and silk moths, can sustain circadian activity rhythms by means of a diffusible signal.

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I. Research Objectives: The objectives entail understanding the functional roles of output signals from the SCN (including diffusible factors) in setting circadian phase of activity rhythms. This involves the identification of pacemaker cells of the SCN, outputs from these cells, and targets of these outputs. Details of these objectives and accomplishments are described below.

2. Status of Effort: The status of research efforts is indicated below in the context of the objectives.

Objective 1. To develop a cell transplantation device that physically isolates grafted embryonic neural cells or cellular aggregates.

This objective has been achieved, and the results are published.

Objective 2. To establish optimal conditions for encapsulated tissue survival in anatomical studies using *in vivo* and *in vitro* techniques using both primary fetal tissue and immortalized SCN cell lines.

This objective has been achieved for *in vivo* studies using primary fetal tissue.

Objective 3. To analyze the functional role in behavioral studies of diffusible factors from encapsulated SCN grafts in setting circadian phase of activity.

This objective has been achieved, and the results are published.

Objective 4. To vary membrane pore size so as to determine the approximate molecular weight range of the critical signals.

This work has been completed. Optimal pore size for copolymer membranes is a statistical feature, with normal distribution. We have shown that these membranes are not the optimal tool for identification of critical pore size of is in progress.

Objective 5. To determine the function of putative pacemaker cells in the hamster.

We have discovered a subnucleus, marked by calbindin-D_{28} (CaBP) positive cells in the core of the hamster SCN. These cells receive light input (Silver et al., 1996). We have shown that ablation of this SCN sub-region abolishes activity rhythms, and that half-SCN transplants that contain these cells restore rhythmicity, while transplants that do not express these cells, do not (J. Neuroscience 1999). In addition, we now have completed data analysis showing a circadian rhythm of nuclear CaBP expression within these cells. This work, taken together, indicates that cells of the calbindin region

are both on the input and output pathway of circadian pacemakers. This data is an important step in the identification of SCN pacemakers, their outputs and their targets.

3. Accomplishments/findings

The encapsulation membranes fabricated from solutions containing Poly(acrylonitrile-vinylchloride) (PAN-PVC) (85%) and polyethylene oxide (PEO) (15%) were tested for restoration of circadian activity rhythms to animals whose own SCN has been ablated. This work shows that the transplanted SCN, like neural pacemakers of Drosophila and silk moths, can sustain circadian activity rhythms by means of a diffusible signal. This work was published in *Nature* (see below).

Location of SCN pacemakers.

Circadian rhythms are fundamental adaptations of organisms to daily periodicities that result from the earth's rotation. In mammals, the anatomical locus of the "biological clock" in the SCN of the brain is well established, and a great deal of interest rests in identifying SCN pacemaker cells, their inputs and outputs. The present proposal focused on the characterization of putative oscillators within the suprachiasmatic nuclei (SCN). It has long been held that SCN neurons are equipotential and that circadian rhythmicity is sustained following ablation, as long as about 25% of the nuclei are spared. Our data present a different view.

We have described a compact subnucleus of calbindin-D28 (CaBP) positive cells in the core of the hamster SCN in which 75% of the cells are fos-positive in response to a light pulse (Silver et al., 1996). This signifies that CaBP cells are on the input pathway to the clock's pacemakers

We have also shown that animals bearing partial lesions of the SCN that spared the subregion delimited by calbindin-d28k (CaBP) cells, sustained circadian locomotor rhythms. Partial lesions that destroy this region but spared other compartments of the SCN resulted in loss of rhythmicity. Next, we have shown that transplants of half SCN punches that contain CaBP cells restore locomotor rhythms in SCN-lesioned host animals, while transplants containing SCN tissue but lacking these cells fail to restore rhythmicity. Taken together, these studies indicate that cells in the region of the CaBP subnucleus of the SCN serve as driving pacemakers for locomotor rhythmicity, and that the SCN is functionally heterogeneous (LeSauter and Silver: Journal of Neuroscience, 1999).

Another objective was to identify the afferent and efferent connections of cells in the CaBP subnucleus. To determine which SCN cells project to known SCN targets, we have injected cholera toxin beta subunit, a retrograde tracer , in the preoptic area (POA), the sub-paraventricular zone, the dorsomedial hypothalamic nucleus (DMH), the paraventricular nucleus of the thalamus. Injections in the PVT and DMH label a large number of cells in the CaBP region. Injections in the POA label a few cells in the CaBP region. CaBP cells do not seem to project to the sub-paraventricular zone. Double label immunocytochemistry has been done to identify the cells projecting to these regions. We have identified connections between the CaBP-ir and other cells of the SCN, using epi- and confocal microscopy and staining for CaBP and vasoactive intestinal peptide (VIP), vasopressin (VP), gastrin releasing peptide (GRP), cholecystokinin (CCK), or neuropeptide Y (NPY), and single label electron microscopy for CaBP. The heaviest terminals on CaBP cells were VIP and NPYñpositive fibers, with less dense GRP, VP and CCK fibers. Dense CaBP-ir terminals were on virtually all of a population of unknown cells interspersed within the SCN CaBP cells and on GRP cells. Additionally, few terminals are seen on most CCK cells. Most VIP and VP cells within the SCN have no CaBP teminals. A few CaBP terminals are seen on a subset of VP cells lying at the border of the SCN and on VIP cells located within or above the optic tract. Single label electron microscopy reveals synapses among CaBP cells. In summary, connections of the CaBP cells of the SCN are highly topographically organized. This work will be presented at the Society for Neuroscience Meeting 1999, and prepared for publication.

4. Personnel supported

Dr. Rae Silver: PI
113-42-0713

Dr. Joseph LeSauter: Research associate
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(Tresco Lab)

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5. Publications

Silver , R., J. LeSauter, P. Tresco, and M.N. Lehman and (1996). A diffusible coupling signal form the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 382: 810-813.

Silver, R., M-T. Romero, H.R. Besmer, R. Leak, J.M. Nunez and J. LeSauter (1996). Calbindin-D_{28k} cells in the hamster SCN express light-induced Fos. NeuroReport 7: 1224-1228.

LeSauter J., Lehman, M.N. and Silver, R. (1996). Restoration of circadian locomotor rhythms by transplants of SCN "micropunches" J. Biol. Rhythms 11: 163-171.

LeSauter, J., Romero, P., Cascio, M. and Silver, R. (1997). Attachement site of grafted SCN influences precision of restored circadian rhythm. J. Biol. Rhythms 12: 327-338.

Silver, R., LeSauter, J., and Lehman, M.N. (*in press)* Contributions of SCN transplant studies to understanding the biological clock. In: Evolution of Circadian Clock (Eds. T. Hiroshige & K. Honma). Hokkaido U. Press, Sapporo.

Silver R. and R.Y. Moore. The suprachiasmatic nucleus and circadian function: an introduction. Chronobiol Int. 15: vii-x, 1998

LeSauter J. and R. Siver. Output signals of the SCN. Chronobiol. Int. 15: 535-550, 1998

Moore R.Y. and R. Silver. Suprachiasmatic nucleus organization. . Chronobiol. Int. 15: 475-487, 1998

Lehman, M., LeSauter, J. and Silver, R. Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. J. Comp. Neurol. 391: 133-145, 1998.

LeSauter, J. and Silver, R. Biological rhythms. Comparative Psychology : A Handbook (Eds. G. Greenberg & M. Haraway). Garland Publishers, NY. 1998.

LeSauter, J. and R. Silver. Localization of an SCN subnucleus regulating locomotor rhythmicity. J. Neurosci. In press.

6. Interactions/Transitions:

a. Participation/presentations at meetings, conferences, seminars
10/01/1996 - 3/31/1999

Silver, R., and J. LeSauter (1996). Parallels between CaBP-ir cells and behavioral phase shifts. Society for Neuroscience Abstract, 22, 2055.

LeSauter J. and R. Silver (1996). Attachment site of grafted SCN influences precision of restored circadian rhythm. Society for Neuroscience Abstract, 22, 2053.

Bryant, D.N., LeSauter, J, Silver, R, Romero, M-T. (1996) Retinal synapses on calbindin-ir cells of the hamster suprachiasmatic nucleus. Society for Neuroscience Abstract, 22:1140.

R. Silver Gordon Conference, August 11-15, 1997, New Hampshire

Silver R., LeSauter J., Bryant, D.N., and Romero, M-T. Connections of calbindin cells of the hamster SCN.Soc. Neurosci. Abstr. 25, 1999.

LeSauter J., Leak R.K., Silver R and Moore R.Y. Hamster suprachiasmatic nucleus: chemoarchitecture and topography of projections.Soc. Neurosci. Abstr. 25, 1999.

LeSauter J., and Silver R. Ontogeny of calbindin-D28K, substance P, and gastrin releasing peptide-positive cells in the core of the hamster SCN.Soc. Neurosci. Abstr. 23, 198.7, 1997.

Silver R., Lehman M.N. and LeSauter J. Use of neurofilaments markers for labelling fiber outgrowth from SCN xenografts. Soc Neurosci Abstr. 23: 522.3, 1997.

b. Consultation at other labs: (Patrick Tresco)

SmithKline Beecham Pharmaceuticals
Cytotherapeutics, Inc.
Becton Dickinson
Ciba Vision

c. Transitions: (Patrick Tresco)
Cell Encapsulation membrane technology

Development of novel drug delivery technology

7. New Discoveries, inventions, patents: None

8. Honors/Awards: (Rae Silver)

Editorial Board

Journal of Comparative Psychology 1985-1989

Journal for Research in Biological Rhythms-1995-1999

Hormones and Behavior 1996-2000

Service to the Scientific Community

President-Elect: Society for Research in Biological Rhythms (term 2000-2002)

Program Chair: International Conference ion Chronobiology, D.C. August 1999.

Co-Chair: NSF-NIH conference on brain-Immune interactions: 1996

Committee of visitors for the Neuroscience Cluster NSF
June 1995.

Chair: External Advisory Committee, NSF Center for the Study of Biological Rhythms, University of Virginia, 1991-2002.

Panel Member, NSF, Undergraduate Education and Instrumentation.
1995

Panel Member, NIMH Psychobiology, Behavior, and Neurosciences,
1994-1996.

Panel Member, NIMH Behavioral Neurosciences
1993-1994.

Panel Member, Sensory & Integrative Systems, NSF,
1987-1989.

Panel Member, Psychobiology, NSF,
1986-1987.

Panel Member, Neuropsychology Panel, NIMH,
1979-1983.

Society for Behavioral Neuroendocrinology, Advisory Board Member 1996-

Program Chair, American Psychological Association
1994

Member-at-Large, American Psychological Association
1994-1997

Member-at-Large: Society for Research in Biological Rhythms,
1994-1996.

Member, Advisory Committee, Society for Research in Biological Rhythms, 1992-1994.

Rapporteur, American Physiological Society, Conference on "Understanding the Biological Clock - from Genetics to Physiology", 1995.

Advisory Committee Member, International Ornithological Society, 1993-1997.

American Ornithological Union, Elective Member, 1994

Member, Search Committee: Director of the Institute of Animal Behavior-Rutgers University, 1989.

Society for Chronobiology, Member at Large 1996

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Final Invention Report March 31 1999

PI Name: Rae Silver

There were no inventions or patents resulting from this research.